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(PATENT)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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In re Patent Application of:  
Toshihiro MORI et al.

Application No.: 10/621,715

Confirmation No.: 1567

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Art Unit: 1634

For: METHOD FOR SEPARATING AND  
PURIFYING A NUCLEIC ACID

Examiner: S. T. Kapushoc

**DECLARATION UNDER 37 C.F.R. § 1.132**

Assistant Commissioner for Patents  
Alexandria, VA 22313-1450

Sir:

I, Yumiko TAKESHITA of 11-46, Senzui 3-chome, Asaka-shi, Saitama, Japan, hereby declare and state that I received a Bachelor degree of Chemical and Biological Sciences from Japan Woman's University on March 1989 and that I have been employed as a worker in research by Fuji Photo Film Co. Ltd. since April 1989.

I declare that I am at present doing research work on nucleic acid extraction and detection in Life Science Research Laboratory of said company.

I am familiar with the subject matter disclosed by the application.

The following experimentation was conducted by me, or under my supervision and control.

**Preparation of Cellulose Microcapsule**

A cellulose microcapsule was prepared in the same manner as in Example 12 of U.S. Patent No. 4,118,336 (Morishita) except for reducing a scale to 1/4. Specially, 10 g of bentonite powder was added to a solution of 2.5 g of cellulose triacetate in 50 ml of methylene chloride and was dispersed. The resulting dispersion was dispersed with propeller stirring to the form of fine droplets into 200 ml of an aqueous solution containing 1 g of sodium laurylbenzene sulfonate dissolved herein. After the methylene chloride was removed by an evaporator to form microcapsules, the microcapsules formed was tried to be filtered, but a clogging of the filter occurred completely. Due to the clogging, various filters such as a glass filter and paper filter were tried, but even in all of the filters, a clogging occurred. Thus, a precipitate was separated, and the remaining was freeze-dried. To the precipitate and the freeze-dried product were added 30 ml of water to be washed three times (a centrifugation with 1500 rpm for 10 minutes and removal of the supernatant was performed three times) to obtain microcapsules encapsulated with cellulose triacetate containing bentonite powder.

Subsequently, 10 g of the microcapsules were swelled for 30 minutes in 6 ml of a mixture of ethanol and water (1:1), and then the supernatant was removed by a centrifugation. The pellet was charged into 6 ml of 0.5 N sodium hydroxide and saponified with stirring at 50°C for 30 minutes. Thereafter, the microcapsules was washed with water three times and then cellulose microcapsule was obtained.

The separation and purification of a plasmid DNA from a recombinant bacterium was performed by using the cellulose microcapsule obtained above.

**Preparation of Solution containing a plasmid DNA**

A frozen recombinant bacterium containing a plasmid: pBlueScript II/GAPDH/DH5 $\alpha$  was thawed at a room temperature, and then the thawed recombinant bacterium was subjected to a tapping of ten times. To the tapped material was added 100  $\mu$ l of RDP dispersion (50mM TrisHCl and 10mM EDTA: pH 8.1) containing 3  $\mu$ l of EDP-A solution (0.3 mg/ml of RNase A and 0.27 mM of sodium acetate) and 1  $\mu$ l of EDP-B solution (0.01 mg/ml of RNase T1 and 32 mM of ammonium sulfate). After the solution was vortexed at a maximum speed for 15 seconds, to the vortexed solution was added 100  $\mu$ l of ADP solution (0.2N sodium hydroxide and 1% SDS: pH 13). Immediately after the addition of ADP, an inversion mixture was slowly performed five times. To the obtained solution was added 140  $\mu$ l of NDP solution (3M potassium acetate and 13.6% acetic acid: pH 5.5). Immediately after the addition of NDP, an inversion mixture was slowly performed five times. The obtained solution was then centrifuged at 18,000g for 10 minutes at room temperature, and then the supernatant was isolated as a solution containing a plasmid DNA (approximately 330  $\mu$ l).

**Isolation of Plasmid DNA**

(1) To 1.7 ml of tube, 70 mg of the cellulose microcapsule obtained above, 320  $\mu$ l of LOP solution (31.3 mM Bis-Tris and 7.1% Tween 20 in 68.5% of ethanol: pH 6.0) and the solution containing a plasmid DNA (approximately 330  $\mu$ l) were added. The obtained solution was subjected to vortexing at a maximum speed for 30 minutes, and then was subjected to a filtration by a glass filter. However, the filtration was impossible due to a clogging, and the plasmid DNA was not able to be isolated.

On the contrary, the method of the present invention can isolate the plasmid DNA by the above-mentioned operation.

(2) Instead to the above operation, the whole solution containing a plasmid DNA (approximately 330  $\mu$ l) after several-time pipetting was added to a tube containing 70 mg of the cellulose microcapsule obtained above. The obtained solution was subjected to vortexing for one minute and centrifuging at 1,000g for one minute, and the supernatant was removed. To the pellet, 750  $\mu$ l of a washing solution (10.8 mM TrisHCl in 80% of ethanol: pH 7.8) was added. The obtained solution was subjected to vortexing for one minute and centrifuging at 1,000g for one minute, and the supernatant was removed. To the pellet, 750  $\mu$ l of the washing solution was added. The obtained solution was subjected to vortexing for one minute and centrifuging at 1,000g for one minute, and the supernatant was removed. To the pellet, 50  $\mu$ l of a recovering solution (10 mM TrisHCl: pH &5) was added, and then the obtained solution was subjected to vortexing for one minute and centrifuging at 1,000g for one minute to obtain the plasmid.

Although a plasmid DNA can be isolated by many further operations in Morishita, the many further operations are an extremely troublesome. Accordingly, from the above result, it can be recognized that the method of the present invention at least has an excellent separation performance.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectively submitted,

Date: \_\_\_\_\_

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Yumiko TAKESHITA